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(54) Title: GROWTH DIFFERENTIATION FACTOR-10

## (57) Abstract

Growth differentiation factor-10 (GDF-10) is disclosed along with its polynucleotide sequence and amino acid sequence. Also disclosed are diagnostic and therapeutic methods of using the GDF-10 polypeptide and polynucleotide sequences.

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## GROWTH DIFFERENTIATION FACTOR-10

### BACKGROUND OF THE INVENTION

#### 1. *Field of the Invention*

The invention relates generally to growth factors and specifically to a  
5 new member of the transforming growth factor beta (TGF- $\beta$ ) superfamily, which is denoted, growth differentiation factor-10 (GDF-10).

#### 2. *Description of Related Art*

The transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily encompasses a group of structurally-related proteins which affect a wide range of  
10 differentiation processes during embryonic development. The family includes, Mullerian inhibiting substance (MIS), which is required for normal male sex development (Behringer, et al., *Nature*, 345:167, 1990), *Drosophila* decapentaplegic (DPP) gene product, which is required for dorsal-ventral axis formation and morphogenesis of the  
15 imaginal disks (Padgett, et al., *Nature*, 325:81-84, 1987), the *Xenopus* Vg-1 gene product, which localizes to the vegetal pole of eggs ((Weeks, et al., *Cell*, 51:861-867, 1987), the activins (Mason, et al., *Biochem. Biophys. Res. Commun.*, 135:957-964, 1986), which can induce the formation of mesoderm and anterior structures in *Xenopus* embryos  
20 (Thomsen, et al., *Cell*, 63:485, 1990), and the bone morphogenetic proteins (BMPs, osteogenin, OP-1) which can induce *de novo* cartilage and bone formation (Sampath, et al., *J. Biol. Chem.*, 265:13198, 1990). The TGF- $\beta$ s can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis, hematopoiesis,

and epithelial cell differentiation (for review, see Massague, *Cell* 49:437, 1987).

The proteins of the TGF- $\beta$  family are initially synthesized as a large precursor protein which subsequently undergoes proteolytic cleavage at 5 a cluster of basic residues approximately 110-140 amino acids from the C-terminus. The C-terminal regions, or mature regions, of the proteins are all structurally related and the different family members can be classified into distinct subgroups based on the extent of their homology. Although the homologies within particular subgroups range from 70% to 10 90% amino acid sequence identity, the homologies between subgroups are significantly lower, generally ranging from only 20% to 50%. In each case, the active species appears to be a disulfide-linked dimer of C-terminal fragments. For most of the family members that have been studied, the homodimeric species has been found to be biologically 15 active, but for other family members, like the inhibins (Ling, *et al.*, *Nature*, 321:779, 1986) and the TGF- $\beta$ s (Cheifetz, *et al.*, *Cell*, 48:409, 1987), heterodimers have also been detected, and these appear to have different biological properties than the respective homodimers.

Identification of new factors that are tissue-specific in their expression 20 pattern will provide a greater understanding of that tissue's development and function and allow development of effective diagnostic and therapeutic regimens.

## SUMMARY OF THE INVENTION

The present invention provides a cell growth and differentiation factor, GDF-10, a polynucleotide sequence which encodes the factor, and antibodies which are immunoreactive with the factor. This factor 5 appears to relate to various cell proliferative disorders, especially those involving those involving uterine, nerve, bone, and adipose tissue.

Thus, in one embodiment, the invention provides a method for detecting a cell proliferative disorder of uterine, nerve, or fat origin and which is associated with GDF-10. In another embodiment, the invention provides 10 a method for treating a cell proliferative disorder by suppressing or enhancing GDF-10 activity.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows expression of GDF-10 mRNA in adult tissues.

5 FIGURE 2 shows nucleotide and predicted amino acid sequence murine GDF-10. Consensus N-glycosylation signals are denoted by plain boxes.

10 FIGURE 3 shows the alignment of the C-terminal sequences of GDF-10 with other members of the TGF- $\beta$  superfamily. The conserved cysteine residues are boxed. Dashes denote gaps introduced in order to maximize alignment.

15 FIGURE 4 shows amino acid homologies with different members of the TGF- $\beta$  superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus.

FIGURE 5 shows an alignment of the C-terminal sequences of human (top lines) and murine (bottom lines) GDF-10.

15 FIGURE 6 shows an autoradiogram of labeled secreted proteins synthesized by 293 cells transfected with a pcDNA1 vector into which the GDF-10 cDNA was inserted in either the antisense (lanes 1 and 2) or sense (lanes 3 and 4) orientation.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a growth and differentiation factor, GDF-10 and a polynucleotide sequence encoding GDF-10. GDF-10 is expressed at highest levels in uterus and fat and at lower levels in other tissues, such as brain. In one embodiment, the invention provides a method for detection of a cell proliferative disorder of uterine, nerve, or fat origin which is associated with GDF-10 expression. In another embodiment, the invention provides a method for treating a cell proliferative disorder by using an agent which suppresses or enhances GDF-10 activity.

The TGF- $\beta$  superfamily consists of multifunctional polypeptides that control proliferation, differentiation, and other functions in many cell types. Many of the peptides have regulatory, both positive and negative, effects on other peptide growth factors. The structural homology between the GDF-10 protein of this invention and the members of the TGF- $\beta$  family, indicates that GDF-10 is a new member of the family of growth and differentiation factors. Based on the known activities of many of the other members, it can be expected that GDF-10 will also possess biological activities that will make it useful as a diagnostic and therapeutic reagent.

The expression of GDF-10 in uterine and fat tissue suggests a variety of applications using the polypeptide, polynucleotide, and antibodies of the invention, related to contraception, fertility, pregnancy, and cell proliferative diseases. Abnormally low levels of the factor may be indicative of impaired function in the uterus while abnormally high levels may be indicative of hypertrophy, hyperplasia, or the presence of ectopic tissue. Hence, GDF-10 may be useful in detecting not only

primary and metastatic neoplasms of uterine origin but in detecting diseases such as endometriosis as well. In addition, GDF-10 may also be useful as an indicator of developmental anomalies in prenatal screening procedures.

5 Several members of the TGF- $\beta$  superfamily possess activities suggesting possible applications for the treatment of cell proliferative disorders, such as cancer. In particular, TGF- $\beta$  has been shown to be potent growth inhibitor for a variety of cell types (Massague, *Cell* 49:437, 1987). MIS has been shown to inhibit the growth of human  
10 endometrial carcinoma tumors in nude mice (Donahoe, *et al.*, *Ann. Surg.* 194:472, 1981), and inhibin  $\alpha$  has been shown to suppress the development of tumors both in the ovary and in the testis (Matzuk, *et al.*, *Nature*, 360:313, 1992). GDF-10 may have similar activity and may therefore be useful as an anti-proliferative agent, such as for the  
15 treatment of endometrial cancer or endometriosis.

Many of the members of the TGF- $\beta$  family are also important mediators of tissue repair. TGF- $\beta$  has been shown to have marked effects on the formation of collagen and causes of striking angiogenic response in the newborn mouse (Roberts, *et al.*, *Proc. Nat'l Acad. Sci., USA* 83:4167, 1986). The BMP's can induce new bone growth and are effective for the treatment of fractures and other skeletal defects (Glowacki, *et al.*, *Lancet*, 1:959, 1981; Ferguson, *et al.*, *Clin. Orthoped. Relat. Res.*, 227:265, 1988; Johnson, *et al.*, *Clin Orthoped Relat. Res.*, 230:257, 1988). Based on the high degree of homology between GDF-10 and BMP-3, GDF-10 may have similar activities and may be useful in repair  
20 of tissue injury caused by trauma or burns for example.  
25

5        GDF-10 may play a role in regulation of the menstrual cycle or regulation of uterine function during pregnancy, and therefore, GDF-10, anti-GDF-10 antibodies, or antisense polynucleotides may be useful either in contraceptive regimens, in enhancing the success of *in vitro* fertilization procedures, or in preventing premature labor.

10      Certain members of this superfamily have expression patterns or possess activities that relate to the function of the nervous system. For example, one family member, namely GDNF, has been shown to be a potent neurotrophic factor that can promote the survival of dopaminergic neurons (Lin, *et al.*, *Science*, 260:1130). Another family member, namely dorsalin, is capable of promoting the differentiation of neural crest cells (Baster, *et al.*, *Cell*, 73:687). The inhibins and activins have been shown to be expressed in the brain (Meunier, *et al.*, *Proc. Nat'l Acad. Sci., USA*, 85:247, 1988; Sawchenko, *et al.*, *Nature*, 334:615, 1988), and activin has been shown to be capable of functioning as a nerve cell survival molecule (Schubert, *et al.*, *Nature*, 344:868, 1990). Another family member, namely GDF-1, is nervous system-specific in its expression pattern (Lee, *Proc. Nat'l Acad. Sci., USA*, 88:4250, 1991), and certain other family members, such as Vgr-1 (Lyons, *et al.*, *Proc. Nat'l Acad. Sci., USA*, 86:4554, 1989; Jones *et al.*, *Development*, 111:581, 1991), OP-1 (Ozkaynak, *et al.*, *J. Biol. Chem.*, 267:25220, 1992), and BMP-4 (Jones, *et al.*, *Development*, 111:531, 1991), are also known to be expressed in the nervous system. By analogy GDF-10 may have applications in the treatment of neurodegenerative diseases or in maintaining cells or tissues in culture prior to transplantation.

25      The expression of GDF-10 in adipose tissue also raises the possibility of applications for GDF-10 in the treatment of obesity or of disorders related to abnormal proliferation of adipocytes. In this regard, TGF- $\beta$

has been shown to be a potent inhibitor of adipocyte differentiation *in vitro* (Ignatz and Massague, *Proc. Natl. Acad. Sci., USA* 82:8530, 1985).

5        The term "substantially pure" as used herein refers to GDF-10 which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify GDF-10 using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the GDF-10 polypeptide can 10      also be determined by amino-terminal amino acid sequence analysis. GDF-10 polypeptide includes functional fragments of the polypeptide, as long as the activity of GDF-10 remains. Smaller peptides containing the biological activity of GDF-10 are included in the invention.

15      The invention provides polynucleotides encoding the GDF-10 protein. These polynucleotides include DNA, cDNA and RNA sequences which encode GDF-10. It is understood that all polynucleotides encoding all or a portion of GDF-10 are also included herein, as long as they encode a polypeptide with GDF-10 activity. Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated 20      polynucleotides. For example, GDF-10 polynucleotide may be subjected to site-directed mutagenesis. The polynucleotide sequence for GDF-10 also includes antisense sequences. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are 25      specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of GDF-10 polypeptide encoded by the nucleotide sequence is functionally unchanged.

Specifically disclosed herein is a cDNA sequence for GDF-10 which is 2322 base pairs in length and contains an open reading frame beginning with a methionine codon at nucleotide 126. The encoded polypeptide is 476 amino acids in length with a molecular weight of about 52.5 kD, as determined by nucleotide sequence analysis. The GDF-10 sequence contains a core of hydrophobic amino acids near the N-terminus, suggestive of a signal sequence for secretion. GDF-10 contains four potential N-glycosylation sites at asparagine residues 114, 152, 277, and 467. GDF-10 contains several potential proteolytic processing sites. Cleavage most likely occurs following arginine 365, which would generate a mature fragment of GDF-10 predicted to be 111 amino acids in length and have an unglycosylated molecular weight of about 12.6kD, as determined by nucleotide sequence analysis. One skilled in the art can modify, or partially or completely remove, the glycosyl groups from the GDF-10 protein using standard techniques. Therefore the functional protein or fragments thereof of the invention includes glycosylated, partially glycosylated and unglycosylated species of GDF-10.

The C-terminal region of GDF-10 following the putative proteolytic processing site shows significant homology to the known members of the TGF- $\beta$  superfamily. The GDF-10 sequence contains most of the residues that are highly conserved in other family members. Among the known family mammalian TGF- $\beta$  family members, GDF-10 is most homologous to BMP-3 (83% sequence identity beginning with the first conserved cysteine residue). GDF-10 also shows significant homology to BMP-3 (approximately 30% sequence identity) in the pro-region of the molecule. Based on these sequence comparisons, GDF-10 and BMP-3 appear to define a new subfamily within the larger superfamily.

Minor modifications of the recombinant GDF-10 primary amino acid sequence may result in proteins which have substantially equivalent activity as compared to the GDF-10 polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or 5 may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity of GDF-10 still exists. Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the 10 development of a smaller active molecule which would have broader utility. For example, one can remove amino or carboxy terminal amino acids which are not required for GDF-10 biological activity.

The nucleotide sequence encoding the GDF-10 polypeptide of the invention includes the disclosed sequence and conservative variations 15 thereof. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for 20 aspartic acid, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with 25 the unsubstituted polypeptide.

DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization techniques which are well known in the art. These include, but are not limited to:

1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences, 2) polymerase chain reaction (PCR) on genomic DNA or cDNA using primers capable of annealing to the DNA sequence of interest, and 3) antibody screening of expression 5 libraries to detect cloned DNA fragments with shared structural features.

Preferably the GDF-10 polynucleotide of the invention is derived from a mammalian organism, and most preferably from a mouse, rat, or human. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, 10 provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic 15 code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded 20 DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic 25 visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, et al., *Nucl. Acid Res.*, 9:879, 1981).

The development of specific DNA sequences encoding GDF-10 can also be obtained by: 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the polypeptide of interest; and 3) *in vitro* synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA.

Of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common. This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of introns.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in

DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, et al., *Nucl. Acid Res.*, 11:2325, 1983).

5 A cDNA expression library, such as lambda gt11, can be screened indirectly for GDF-10 peptides having at least one epitope, using antibodies specific for GDF-10. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of GDF-10 cDNA.

10 DNA sequences encoding GDF-10 can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the 15 term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

20 In the present invention, the GDF-10 polynucleotide sequences may be inserted into a recombinant expression vector. The term "recombinant expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the GDF-10 genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically 25 contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg,

et al., *Gene*, 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.*, 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (e.g., T7, metallothionein I, or polyhedrin promoters).

Polynucleotide sequences encoding GDF-10 can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention. Preferably, the mature C-terminal region of GDF-10 is expressed from a cDNA clone containing the entire coding sequence of GDF-10. Alternatively, the C-terminal portion of GDF-10 can be expressed as a fusion protein with the pro- region of another member of the TGF- $\beta$  family or co-expressed with another pro- region (see for example, Hammonds, et al., *Molec. Endocrin.* 5:149, 1991; Gray, A., and Mason, A., *Science*, 247:1328, 1990).

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the  $\text{CaCl}_2$  method using procedures well known in the art. Alternatively,  $\text{MgCl}_2$  or  $\text{RbCl}$  can be used. Transformation can also be performed after forming a protoplast of the host cell if desired.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also 5 be cotransformed with DNA sequences encoding the GDF-10 of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform 10 eukaryotic cells and express the protein. (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and 15 immunological separations involving monoclonal or polyclonal antibodies.

The invention includes antibodies immunoreactive with GDF-10 polypeptide or functional fragments thereof. Antibody which consists essentially of pooled monoclonal antibodies with different epitopic 20 specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art (Kohler, et al., *Nature*, 256:495, 1975). The term antibody as used in this invention is meant to include intact molecules as well as 25 fragments thereof, such as Fab and F(ab')<sub>2</sub>, which are capable of binding an epitopic determinant on GDF-10.

The term "cell-proliferative disorder" denotes malignant as well as non-malignant cell populations which often appear to differ from the surrounding tissue both morphologically and genotypically. The term "cell-proliferative disorder" also includes situations in which a normally occurring process could be enhanced or suppressed for clinical benefit; an example of such a process would be fracture healing. Malignant cells (i.e. cancer) develop as a result of a multistep process. The GDF-10 polynucleotide that is an antisense molecule is useful in treating malignancies of the various organ systems, particularly, for example, 5 cells in uterine or adipose tissue. Essentially, any disorder which is etiologically linked to altered expression of GDF-10 could be considered 10 susceptible to treatment with a GDF-10 suppressing reagent. One such disorder is a malignant cell proliferative disorder, for example.

The invention provides a method for detecting a cell proliferative disorder of uterine or adipose tissue which comprises contacting an 15 anti-GDF-10 antibody with a cell suspected of having a GDF-10 associated disorder and detecting binding to the antibody. The antibody reactive with GDF-10 is labeled with a compound which allows detection 20 of binding to GDF-10. For purposes of the invention, an antibody specific for GDF-10 polypeptide may be used to detect the level of GDF-10 in biological fluids and tissues. Any specimen containing a detectable amount of antigen can be used. A preferred sample of this 25 invention is uterine or fat tissue. The level of GDF-10 in the suspect cell can be compared with the level in a normal cell to determine whether the subject has a GDF-10-associated cell proliferative disorder. Preferably the subject is human.

The antibodies of the invention can be used in any subject in which it is desirable to administer *in vitro* or *in vivo* immunodiagnosis or

immunotherapy. The antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

15 The antibodies of the invention can be bound to many different carriers and used to detect the presence of an antigen comprising the polypeptide of the invention. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation.

20

25 There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds. Those

of ordinary skill in the art will know of other suitable labels for binding to the antibody, or will be able to ascertain such, using routine experimentation.

Another technique which may also result in greater sensitivity consists 5 of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts with avidin, or dinitrophenyl, puridoxal, and fluorescein, which can react with specific antihapten antibodies.

10 In using the monoclonal antibodies of the invention for the *in vivo* detection of antigen, the detectably labeled antibody is given a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled monoclonal antibody is administered in sufficient quantity to enable detection of the site having 15 the antigen comprising a polypeptide of the invention for which the monoclonal antibodies are specific.

The concentration of detectably labeled monoclonal antibody which is administered should be sufficient such that the binding to those cells having the polypeptide is detectable compared to the background. 20 Further, it is desirable that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.

As a rule, the dosage of detectably labeled monoclonal antibody for *in vivo* diagnosis will vary depending on such factors as age, sex, and 25 extent of disease of the individual. Such dosages may vary, for

example, depending on whether multiple injections are given, antigenic burden, and other factors known to those of skill in the art.

For *in vivo* diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor in selecting a radioisotope for *in vivo* diagnosis is that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for *in vivo* imaging will lack a particle emission, but produce a large number of photons in the 140-250 keV range, which may readily be detected by conventional gamma cameras.

For *in vivo* diagnosis radioisotopes may be bound to immunoglobulin either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bifunctional chelating agents such as diethylenetriaminepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are <sup>111</sup>In, <sup>97</sup>Ru, <sup>67</sup>Ga, <sup>68</sup>Ga, <sup>72</sup>As, <sup>89</sup>Zr, and <sup>201</sup>Tl.

The monoclonal antibodies of the invention can also be labeled with a paramagnetic isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements

which are particularly useful in such techniques include  $^{157}\text{Gd}$ ,  $^{55}\text{Mn}$ ,  $^{162}\text{Dy}$ ,  $^{52}\text{Cr}$ , and  $^{56}\text{Fe}$ .

The monoclonal antibodies of the invention can be used *in vitro* and *in vivo* to monitor the course of amelioration of a GDF-10-associated disease in a subject. Thus, for example, by measuring the increase or decrease in the number of cells expressing antigen comprising a polypeptide of the invention or changes in the concentration of such antigen present in various body fluids, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the GDF-10-associated disease is effective. The term "ameliorate" denotes a lessening of the detrimental effect of the GDF-10-associated disease in the subject receiving therapy.

The present invention identifies a nucleotide sequence that can be expressed in an altered manner as compared to expression in a normal cell, therefore it is possible to design appropriate therapeutic or diagnostic techniques directed to this sequence. Thus, where a cell-proliferative disorder is associated with the expression of GDF-10, nucleic acid sequences that interfere with GDF-10 expression at the translational level can be used. This approach utilizes, for example, antisense nucleic acid and ribozymes to block translation of a specific GDF-10 mRNA, either by masking that mRNA with an antisense nucleic acid or by cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense nucleic acids interfere with

the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced into the target 5 GDF-10-producing cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal.Biochem.*, 172:289, 1988).

10 Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J.Amer.Med. Assn.*, 260:3030, 1988). A major advantage of this approach is that, because they are sequence-15 specific, only mRNAs with particular sequences are inactivated.

20 There are two basic types of ribozymes namely, *tetrahymena*-type (Hasselhoff, *Nature*, 334:585, 1988) and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a specific 25 mRNA species and 18-based recognition sequences are preferable to shorter recognition sequences.

The present invention also provides gene therapy for the treatment of cell proliferative disorders which are mediated by GDF-10 protein. Such

therapy would achieve its therapeutic effect by introduction of the GDF-10 antisense polynucleotide into cells having the proliferative disorder. Delivery of antisense GDF-10 polynucleotide can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Especially preferred for therapeutic delivery of antisense sequences is the use of targeted liposomes.

Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting a GDF-10 sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target specific. Retroviral vectors can be made target specific by inserting, for example, a polynucleotide encoding a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome to allow target specific delivery of the retroviral vector containing the GDF-10 antisense polynucleotide.

Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsidation. Helper cell lines which have deletions of the packaging signal include, but are not limited to  $\Psi$ 2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but the structural genes are replaced by other genes of interest, the vector can be packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes *gag*, *pol* and *env*, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for GDF-10 antisense polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0  $\mu$ m can encapsulate a substantial percentage of an aqueous

buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., *Trends Biochem. Sci.*, 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, et al., *Biotechniques*, 6:682, 1988).

The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon 5 whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by 10 coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

15 The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

20 Due to the expression of GDF-10 primarily in uterine and adipose tissue, there are a variety of applications using the polypeptide, polynucleotide, and antibodies of the invention, related to these and other tissues. Such applications include treatment of cell proliferative disorders involving these and other tissues, including bone. In addition, GDF-10 may be useful in various gene therapy procedures.

25 The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

**EXAMPLE 1**  
**IDENTIFICATION AND ISOLATION OF A NOVEL**  
**TGF- $\beta$  FAMILY MEMBER**

To identify new members of the TGF- $\beta$  superfamily, degenerate  
5 oligonucleotides were designed which corresponded to two conserved  
regions among the known family members: one region downstream of  
the first conserved cysteine residue and the other region spanning the  
invariant cysteine residues near the C-terminus. These primers were  
used for polymerase chain reactions on lung and brain cDNA followed  
10 by subcloning the PCR products using restriction sites placed at the 5'  
ends of the primers, picking individual *E. coli* colonies carrying these  
subcloned inserts, and using a combination of random sequencing and  
hybridization analysis to eliminate known members of the superfamily.

15 GDF-10 was identified from a mixture of PCR products obtained with the  
primers:

NSC1: 5'-

CCGGAATTCAA(G/A)GT(G/A/T/C)GA(T/C)TT(T/C)GC(G/A/T/C)GA  
(T/C)AT(A/C/T)GG(G/A/T/C)TGG-3'

NSC2: 5'-

20 CCGGAATTCA(A/G)CA(G/A/T/C)GC(A/G)CA(G/A)CT(T/C)TC(G/A/T/C)  
AC(G/A/T/C)GTCAT-3'

NSC3: 5'-

CCGGAATTCA(A/G)CA(G/A/T/C)GC(A/G)CA(G/A/T/C)GA(T/C)TC  
(G/A/T/C)AC(G/A/T/C)GTCAT-3'

25 PCR using primers NSC1 with NSC2 or NSC1 with NSC3 was carried  
out with cDNA prepared from 0.25  $\mu$ g of lung or brain mRNA for 35  
cycles at 94°C for 1 min, 50°C for 2 min, and 72°C for 2 min. PCR  
products of approximately 300 base pairs were digested with Eco RI, gel  
purified, and subcloned in the Bluescript vector (Stratagene, San Diego,

CA). DNA was prepared from bacterial colonies carrying individual subclones and sequenced. Of 11 clones that were sequenced, 9 corresponded to BMP-3, and two represented a novel sequence, which was designated GDF-10.

5

## EXAMPLE 2

### EXPRESSION PATTERN AND SEQUENCE OF GDF-10

10 To determine the expression pattern of GDF-10, RNA samples prepared from a variety of adult tissues were screened by Northern analysis. 2.5 micrograms of twice polyA-selected RNA prepared from each tissue were electrophoresed on formaldehyde gels, blotted and probed with GDF-10. As shown in Figure 1, the GDF-10 probe detected an mRNA expressed at highest levels in uterus, fat, and brain.

15 A murine uterus cDNA library consisting of  $3 \times 10^6$  recombinant phage was constructed in lambda ZAP II and screened with a probe derived from the GDF-10 PCR product. The entire nucleotide sequence of the longest of 7 hybridizing clones is shown in Figure 2. Consensus N-glycosylation signals are denoted by plain boxes. Numbers indicate nucleotide position relative to the 5' end. The 2322 bp sequence contains a long open reading frame beginning with a methionine codon at nucleotide 126 and potentially encoding a protein 476 amino acids in length with a molecular weight of 52.5 kD. The predicted GDF-10 amino acid sequence contains a hydrophobic N-terminal region, suggestive of a signal sequence for secretion, four potential N-linked glycosylation sites at asparagine residues 114, 152, 277, and 467 and a putative proteolytic processing site at amino acid 365. Cleavage of the GDF-10 precursor at this site would generate a mature GDF-10

protein 111 amino acids in length with a predicted unglycosylated molecular weight of 12.6 kD.

The C-terminal region of GDF-10 following the putative proteolytic processing site shows significant homology to the known members of the TGF- $\beta$  superfamily (Figure 3). Figure 3 shows the alignment of the C-terminal sequences of GDF-10 with the corresponding regions of human GDF-1 (Lee, *Proc. Natl. Acad. Sci. USA*, 88:4250-4254, 1991), murine GDF-3 and GDF-9 (McPherron and Lee, *J. Biol. Chem.*, 268:3444, 1993), human BMP-2 and 4 (Wozney, et al., *Science*, 242:1528-1534, 1988), human Vgr-1 (Celeste, et al., *Proc. Natl. Acad. Sci. USA*, 87:9843-9847, 1990), human OP-1 (Ozkaynak, et al., *EMBO J.*, 9:2085-2093, 1990), human BMP-5 (Celeste, et al., *Proc. Natl. Acad. Sci. USA*, 87:9843-9847, 1990), human OP-2 (Ozkaynak, et al., *J. Biol. Chem.*, 267:25220-25227, 1992), human BMP-3 (Wozney, et al., *Science*, 242:1528-1534, 1988), human MIS (Cate, et al., *Cell*, 45:685-698, 1986), human inhibin alpha,  $\beta$ A, and  $\beta$ B (Mason, et al., *Biochem. Biophys. Res. Commun.*, 135:957-964, 1986), murine nodal (Zhou, et al., *Nature*, 361:543-547, 1993), human TGF- $\beta$ 1 (Derynck, et al., *Nature*, 316:701-705, 1985), human TGF- $\beta$ 2 (deMartin, et al., *EMBO J.*, 6:3673-3677, 1987), and human TGF- $\beta$ 3 (ten Dijke, et al., *Proc. Natl. Acad. Sci. USA*, 85:4715-4719, 1988). The conserved cysteine residues are boxed. Dashes denote gaps introduced in order to maximize the alignment.

GDF-10 contains most of the residues that are highly conserved in other family members, including the seven cysteine residues with their characteristic spacing.

5 FIGURE 4 shows the amino acid homologies among the different members of the TGF- $\beta$  superfamily. Numbers represent percent amino acid identities calculated from the first conserved cysteine to the C-terminus. In this region, GDF-10 is most homologous to BMP-3 (83% sequence identity).

**EXAMPLE 3**  
**ISOLATION OF HUMAN GDF-10**

10 To isolate human GDF-10, a human uterus cDNA library consisting of  $16.2 \times 10^6$  recombinant phage was constructed in lambda ZAP II and screened with a murine GDF-10 probe. From this library, 20 hybridizing clones were isolated. Partial nucleotide sequence analysis of the longest clone showed that human and murine GDF-10 are highly homologous; the predicted amino acid sequences are 97% identical beginning with the first conserved cysteine residue following the 15 predicted cleavage site (Figure 5).

**EXAMPLE 4**  
**SECRETION OF GDF-10 BY MAMMALIAN CELLS**

20 To determine whether GDF-10 is secreted by mammalian cells, the GDF-10 cDNA was cloned into the pcDNA1 expression vector and transfected into 293 cells. Following DNA transfection, the cells were metabolically labeled with a mixture of [ $^{35}$ S]-cysteine and [ $^{35}$ S]-methionine, and labeled secreted proteins were analyzed by SDS-polyacrylamide gel electrophoresis. As shown in Figure 6, additional bands were detected in cells transfected with a sense GDF-10 construct 25 compared to an antisense control construct. The presence of multiple

protein species most likely indicates that 293 cells are capable of proteolytically processing GDF-10. Hence, these data suggest that GDF-10 is secreted by these cells and that GDF-10 is cleaved, as predicted from the cDNA sequence.

- 5 Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

(ii) TITLE OF INVENTION: GROWTH DIFFERENTIATION FACTOR-10

5 (iii) NUMBER OF SEQUENCES: 26

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10 (C) CITY: Los Angeles

(D) STATE: California

(E) COUNTRY: USA

(F) ZIP: 90067

## (v) COMPUTER READABLE FORM:

15 (A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

## (vi) CURRENT APPLICATION DATA:

20 (A) APPLICATION NUMBER: PCT

(B) FILING DATE: 07-OCT-1994

(C) CLASSIFICATION:

## (viii) ATTORNEY/AGENT INFORMATION:

25 (A) NAME: LISA A. HAILE, PH.D.

(B) REGISTRATION NUMBER: P-38,347

(C) REFERENCE/DOCKET NUMBER: FD-3054 PCT

## (ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (619) 455-5100

(B) TELEFAX: (619) 455-5110

## 30 (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

-32-

(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

5 (vii) IMMEDIATE SOURCE:  
(B) CLONE: NSC1

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 1..36

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

10 CCGGAATTCA ARGTNGAYTT YGCNGAYATH GGNTGG 36

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 33 base pairs  
(B) TYPE: nucleic acid  
15 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:  
(B) CLONE: NSC2

20 (ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 1..33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCGGAATTCA CANGCRCARC TYTCNACNGT CAT

33

25 (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 33 base pairs

-33-

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

5 (vii) IMMEDIATE SOURCE:

- (B) CLONE: NSC3

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..33

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCGGAATTCA CANGCRCANG AYTCNACNGT CAT

33

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2322 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

15 (B) CLONE: Murine GDF-10

(ix) FEATURE:

- (A) NAME/KEY: CDS

(B) LOCATION: 126..1553

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

	TGGGGTCATC CGGGCTGTCC GAGTCCCACA GGGACAACTC CAGCCGCGGA CGAGGTGCAC	60
	AGCCAAACACT GAGCCCTCCT TGTCTGTTCT CCTGGGCTCA GACCCTTCAC CACCGTTACT	120
	CAGCC ATG GCT CCA GGT CCT GCT CGG ATC AGC TTG GGG TCC CAG CTG	167
5	Met Ala Pro Gly Pro Ala Arg Ile Ser Leu Gly Ser Gln Leu	
	1 5 10	
	CTG CCC ATG GTG CCG CTG CTC CTG CTG CGG GGC GCA GGC TGC GGC	215
	Leu Pro Met Val Pro Leu Leu Leu Leu Arg Gly Ala Gly Cys Gly	
	15 20 25 30	
10	CAC AGG GGC CCC TCA TGG TCC TCA TTG CCC TCG GCA GCT GCC GGT CTG	263
	His Arg Gly Pro Ser Trp Ser Ser Leu Pro Ser Ala Ala Ala Gly Leu	
	35 40 45	
	CAG GGG GAC AGG GAC TCC CAG CAG TCA CCC GGG GAC GCA GCA GCC GCT	311
15	Gln Gly Asp Arg Asp Ser Gln Gln Ser Pro Gly Asp Ala Ala Ala Ala	
	50 55 60	
	CTG GGC CCA GGC GCC CAG GAC ATG GTC GCT ATC CAC ATG CTC AGG CTC	359
	Leu Gly Pro Gly Ala Gln Asp Met Val Ala Ile His Met Leu Arg Leu	
	65 70 75	
20	TAT GAG AAG TAC AAC CGA AGA GGT GCT CCA CCG GGA GGA GGC AAC ACC	407
	Tyr Glu Lys Tyr Asn Arg Arg Gly Ala Pro Pro Gly Gly Asn Thr	
	80 85 90	
	GTC CGA AGC TTC CGT GCC CGG CTG GAA ATG ATC GAC CAA AAG CCT GTG	455
	Val Arg Ser Phe Arg Ala Arg Leu Glu Met Ile Asp Gln Lys Pro Val	
	95 100 105 110	
25	TAT TTC TTC AAC TTG ACT TCC ATG CAA GAC TCA GAA ATG ATC CTC ACA	503
	Tyr Phe Phe Asn Leu Thr Ser Met Gln Asp Ser Glu Met Ile Leu Thr	
	115 120 125	
	GCC GCC TTC CAC TTC TAC TCA GAA CCT CCA CGG TGG CCC CGG GCT GGT	551
	Ala Ala Phe His Phe Tyr Ser Glu Pro Pro Arg Trp Pro Arg Ala Gly	
30	130 135 140	
	GAG GTA TTC TGC AAG CCC CGA GCT AAG AAC GCA TCC TGC CGC CTC CTG	599
	Glu Val Phe Cys Lys Pro Arg Ala Lys Asn Ala Ser Cys Arg Leu Leu	
	145 150 155	

	ACC CCA GGG CTG CCT GCA CGC TTG CAC CTA ATC TTC CGC AGT CTT TCC	647
	Thr Pro Gly Leu Pro Ala Arg Leu His Leu Ile Phe Arg Ser Leu Ser	
	160 165 170	
5	CAG AAC ACC GCC ACT CAG GGG CTG CTC CGC GGG GCC ATG GCC CTG ACG	695
	Gln Asn Thr Ala Thr Gln Gly Leu Leu Arg Gly Ala Met Ala Leu Thr	
	175 180 185 190	
	CCT CCA CCA CGT GGC CTG TGG CAG GCC AAG GAC ATC TCC TCA ATC ATC	743
	Pro Pro Pro Arg Gly Leu Trp Gln Ala Lys Asp Ile Ser Ser Ile Ile	
	195 200 205	
10	AAG GCT GCC CGA AGG GAT GGA GAG CTG CTT CTC TCT GCT CAG CTG GAT	791
	Lys Ala Ala Arg Arg Asp Gly Glu Leu Leu Leu Ser Ala Gln Leu Asp	
	210 215 220	
15	ACT GGG GAG AAG GAC CCC GGA GTG CCA CGG CCC AGT TCC CAC ATG CCC	839
	Thr Gly Glu Lys Asp Pro Gly Val Pro Arg Pro Ser Ser His Met Pro	
	225 230 235	
	TAT ATC CTT GTC TAC GCC AAT GAC CTG GCC ATC TCC GAA CCC AAC AGT	887
	Tyr Ile Leu Val Tyr Ala Asn Asp Leu Ala Ile Ser Glu Pro Asn Ser	
	240 245 250	
20	GTA GCA GTG TCG CTA CAG AGA TAC GAC CCA TTT CCA GCT GGA GAC TTT	935
	Val Ala Val Ser Leu Gln Arg Tyr Asp Pro Phe Pro Ala Gly Asp Phe	
	255 260 265 270	
	GAG CCT GGA GCA GCC CCC AAC AGC TCA GCT GAT CCC CGC GTG CGC AGG	983
	Glu Pro Gly Ala Ala Pro Asn Ser Ser Ala Asp Pro Arg Val Arg Arg	
	275 280 285	
25	GCG GCT CAG GTG TCA AAA CCC CTG CAA GAC AAT GAA CTG CCG GGG CTG	1031
	Ala Ala Gln Val Ser Lys Pro Leu Gln Asp Asn Glu Leu Pro Gly Leu	
	290 295 300	
30	GAT GAA AGA CCA GCG CCT GCC CTG CAT GCC CAG AAT TTC CAC AAG CAC	1079
	Asp Glu Arg Pro Ala Pro Ala Leu His Ala Gln Asn Phe His Lys His	
	305 310 315	
	GAG TTC TGG TCC AGT CCT TTC CGG GCA CTG AAA CCC CGC ACG GCG CGC	1127
	Glu Phe Trp Ser Ser Pro Phe Arg Ala Leu Lys Pro Arg Thr Ala Arg	
	320 325 330	

-36-

	AAA GAC CGC AAG AAG AAG GAC CAG GAC ACA TTC ACC GCC GCC TCC TCT	1175
	Lys Asp Arg Lys Lys Asp Gln Asp Thr Phe Thr Ala Ala Ser Ser	
	335 340 345 350	350
5	CAG GTG CTG GAC TTT GAC GAG AAG ACG ATG CAG AAA GCC AGG AGG CGG	1223
	Gln Val Leu Asp Phe Asp Glu Lys Thr Met Gln Lys Ala Arg Arg Arg	
	355 360 365	
	CAG TGG GAT GAG CCC CGG GTC TGC TCC AGG AGG TAC CTG AAG GTG GAT	1271
	Gln Trp Asp Glu Pro Arg Val Cys Ser Arg Arg Tyr Leu Lys Val Asp	
	370 375 380	
10	TTT GCA GAC ATC GGG TGG AAT GAA TGG ATC ATC TCT CCC AAA TCC TTT	1319
	Phe Ala Asp Ile Gly Trp Asn Glu Trp Ile Ile Ser Pro Lys Ser Phe	
	385 390 395	
15	GAC GCC TAC TAC TGT GCT GGG GCC TGC GAG TTC CCC ATG CCC AAG ATT	1367
	Asp Ala Tyr Tyr Cys Ala Gly Ala Cys Glu Phe Pro Met Pro Lys Ile	
	400 405 410	
	GTC CGC CCA TCC AAC CAT GCC ACC ATC CAG AGC ATC GTC AGA GCT GTG	1415
	Val Arg Pro Ser Asn His Ala Thr Ile Gln Ser Ile Val Arg Ala Val	
	415 420 425 430	
20	GGC ATT GTC CCT GGC ATC CCA GAG CCA TGC TGT GTT CCA GAC AAG ATG	1463
	Gly Ile Val Pro Gly Ile Pro Glu Pro Cys Cys Val Pro Asp Lys Met	
	435 440 445	
	AAC TCC CTT GGA GTC CTT TTC CTG GAT GAA AAT CGG AAT GCG GTT CTG	1511
	Asn Ser Leu Gly Val Leu Phe Leu Asp Glu Asn Arg Asn Ala Val Leu	
	450 455 460	
25	AAG GTG TAC CCC AAT ATG TCC GTA GAG ACC TGT GCC TGT CGG	1553
	Lys Val Tyr Pro Asn Met Ser Val Glu Thr Cys Ala Cys Arg	
	465 470 475	
	TAAGATGGCT TCAAGATAGA AGACAGACCT GCTTCATCCC TGCCCTGCAG AGTGGCAATC	1613
	TTGGAGCCAG GGACTTGACT CGGGGAGGTT CCAGGTGCTA GACAGAGCTT ACAGGCAGCC	1673
30	CTGCTGGGAC CAAGAAAGAT CTGCCACCA CATCGCAATT CTTCAGTTCT TCCGTGCTGG	1733
	TGGTAGCTCT GTAAAGACGT GTTGAGTTCC TGGAAGAAAT CTGGAATTAA CTGTGGTCTG	1793
	CAATTTGCCCTG CCCACACTTT TCAAGGCCTA GAAATAACGT GTGTCCTCAA	1853

ATGTCAACTC CAGGCATTG TCCTCTCAAA ACCTAGAAAG ACTATGCAAA TCTTGGGTA 1913  
 CTCCCCCCCC CCATGGCAGT TTAAATGCTG TTTTAAAACC CTCAGGCTGC ATTCTAGAAA 1973  
 CAGGGCCTAA CCCATGGCAC GAGTGAGTAT TTTCTTTAC GTTTCACTAC ACGTGCTTT 2033  
 ATACATGCAG TATGCACATG TAATCACGGT TGATTTCTTC TTTTAATATA TGTATTTCTA 2093  
 5 TTTCAAAGCA AACGGAGAG AGTCGATCCC ATCCCCTGCA GAGGTAATAA TGCAAGTTAG 2153  
 GTGTGGGTTG TCTAAGCATG TGTATGGAAA TAATACATAC AGTAATATGC TGGAATACTA 2213  
 AAAAAGTAAC CAAGATTTA TATTTTGTA AATTATACTT TGTATACTGT AGATTGTGAG 2273  
 TGTTCTGTGT TTTTATGGAA AGCTAATAAA TTAAAGGTGC GGAGGTATC 2322

## (2) INFORMATION FOR SEQ ID NO:5:

## 10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 476 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met	Ala	Pro	Gly	Pro	Ala	Arg	Ile	Ser	Leu	Gly	Ser	Gln	Leu	Leu	Pro
1															15
5															
Met	Val	Pro	Leu	Leu	Leu	Leu	Leu	Arg	Gly	Ala	Gly	Cys	Gly	His	Arg
	20														30
25															
Gly	Pro	Ser	Trp	Ser	Ser	Leu	Pro	Ser	Ala	Ala	Ala	Gly	Leu	Gln	Gly
	35														45
40															
Asp	Arg	Asp	Ser	Gln	Gln	Ser	Pro	Gly	Asp	Ala	Ala	Ala	Leu	Gly	
	50														60
55															
Pro	Gly	Ala	Gln	Asp	Met	Val	Ala	Ile	His	Met	Leu	Arg	Leu	Tyr	Glu
	65														80
70															
Lys	Tyr	Asn	Arg	Arg	Gly	Ala	Pro	Pro	Gly	Gly	Gly	Asn	Thr	Val	Arg
	85														95
90															

-38-

Ser Phe Arg Ala Arg Leu Glu Met Ile Asp Gln Lys Pro Val Tyr Phe  
100 105 110

Phe Asn Leu Thr Ser Met Gln Asp Ser Glu Met Ile Leu Thr Ala Ala  
115 120 125

5 Phe His Phe Tyr Ser Glu Pro Pro Arg Trp Pro Arg Ala Gly Glu Val  
130 135 140

Phe Cys Lys Pro Arg Ala Lys Asn Ala Ser Cys Arg Leu Leu Thr Pro  
145 150 155 160

Gly Leu Pro Ala Arg Leu His Leu Ile Phe Arg Ser Leu Ser Gln Asn  
10 165 170 175

Thr Ala Thr Gln Gly Leu Leu Arg Gly Ala Met Ala Leu Thr Pro Pro  
180 185 190

Pro Arg Gly Leu Trp Gln Ala Lys Asp Ile Ser Ser Ile Ile Lys Ala  
195 200 205

15 Ala Arg Arg Asp Gly Glu Leu Leu Leu Ser Ala Gln Leu Asp Thr Gly  
210 215 220

Glu Lys Asp Pro Gly Val Pro Arg Pro Ser Ser His Met Pro Tyr Ile  
225 230 235 240

Leu Val Tyr Ala Asn Asp Leu Ala Ile Ser Glu Pro Asn Ser Val Ala  
20 245 250 255

Val Ser Leu Gln Arg Tyr Asp Pro Phe Pro Ala Gly Asp Phe Glu Pro  
260 265 270

Gly Ala Ala Pro Asn Ser Ser Ala Asp Pro Arg Val Arg Arg Ala Ala  
275 280 285

25 Gln Val Ser Lys Pro Leu Gln Asp Asn Glu Leu Pro Gly Leu Asp Glu  
290 295 300

Arg Pro Ala Pro Ala Leu His Ala Gln Asn Phe His Lys His Glu Phe  
305 310 315 320

Trp Ser Ser Pro Phe Arg Ala Leu Lys Pro Arg Thr Ala Arg Lys Asp  
30 325 330 335

-39-

Arg Lys Lys Lys Asp Gln Asp Thr Phe Thr Ala Ala Ser Ser Gln Val  
340 345 350

Leu Asp Phe Asp Glu Lys Thr Met Gln Lys Ala Arg Arg Arg Gln Trp  
355 360 365

5 Asp Glu Pro Arg Val Cys Ser Arg Arg Tyr Leu Lys Val Asp Phe Ala  
370 375 380

Asp Ile Gly Trp Asn Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala  
385 390 395 400

Tyr Tyr Cys Ala Gly Ala Cys Glu Phe Pro Met Pro Lys Ile Val Arg  
10 405 410 415

Pro Ser Asn His Ala Thr Ile Gln Ser Ile Val Arg Ala Val Gly Ile  
420 425 430

Val Pro Gly Ile Pro Glu Pro Cys Cys Val Pro Asp Lys Met Asn Ser  
435 440 445

15 Leu Gly Val Leu Phe Leu Asp Glu Asn Arg Asn Ala Val Leu Lys Val  
450 455 460

Tyr Pro Asn Met Ser Val Glu Thr Cys Ala Cys Arg  
465 470 475

(2) INFORMATION FOR SEQ ID NO:6:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 120 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:  
(B) CLONE: GDF-10

(ix) FEATURE:  
(A) NAME/KEY: Protein  
30 (B) LOCATION: 1..120

-40-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Glu Lys Ser Met Gln Lys Ala Arg Arg Arg Gln Trp Asp Glu Pro Arg  
1 5 10 15

Val Cys Ser Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp  
5 20 25 30

Asn Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ala  
35 40 45

Gly Ala Cys Glu Phe Pro Met Pro Lys Ile Val Arg Pro Ser Asn His  
50 55 60

10 Ala Thr Ile Gln Ser Ile Val Arg Ala Val Gly Ile Val Pro Gly Ile  
65 70 75 80

Pro Glu Pro Cys Cys Val Pro Asp Lys Met Asn Ser Leu Gly Val Leu  
85 90 95

15 Phe Leu Asp Glu Asn Arg Asn Ala Val Leu Lys Val Tyr Pro Asn Met  
100 105 110

Ser Val Glu Thr Cys Ala Cys Arg  
115 120

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 123 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:

(B) CLONE: GDF-1

(ix) FEATURE:

(A) NAME/KEY: Protein  
(B) LOCATION: 1..123

-41-

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

	Arg	Pro	Arg	Arg	Asp	Ala	Glu	Pro	Val	Leu	Gly	Gly	Gly	Pro	Gly	Gly
1						5				10				15		
5																
	Ala	Cys	Arg	Ala	Arg	Arg	Leu	Tyr	Val	Ser	Phe	Arg	Glu	Val	Gly	Trp
							20			25				30		
	His	Arg	Trp	Val	Ile	Ala	Pro	Arg	Gly	Phe	Leu	Ala	Asn	Tyr	Cys	Gln
							35			40			45			
	Gly	Gln	Cys	Ala	Leu	Pro	Val	Ala	Leu	Ser	Gly	Ser	Gly	Gly	Pro	Pro
						50			55				60			
10																
	Ala	Leu	Asn	His	Ala	Val	Leu	Arg	Ala	Leu	Met	His	Ala	Ala	Ala	Pro
						65			70			75			80	
	Gly	Ala	Ala	Asp	Leu	Pro	Cys	Cys	Val	Pro	Ala	Arg	Leu	Ser	Pro	Ile
							85			90			95			
15																
	Ser	Val	Leu	Phe	Phe	Asp	Asn	Ser	Asp	Asn	Val	Val	Leu	Arg	Gln	Tyr
							100			105			110			
	Glu	Asp	Met	Val	Val	Asp	Glu	Cys	Gly	Cys	Arg					
						115			120							

## (2) INFORMATION FOR SEQ ID NO:8:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 118 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:

- (B) CLONE: GDF-3

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..118

-42-

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Arg Lys Arg Arg Ala Ala Ile Ser Val Pro Lys Gly Phe Cys Arg Asn  
1 5 10 15

Phe Cys His Arg His Gln Leu Phe Ile Asn Phe Gln Asp Leu Gly Trp  
5 20 25 30

His Lys Trp Val Ile Ala Pro Lys Gly Phe Met Ala Asn Tyr Cys His  
35 40 45

Gly Glu Cys Pro Phe Ser Met Thr Thr Tyr Leu Asn Ser Ser Asn Tyr  
50 55 60

10 Ala Phe Met Gln Ala Leu Met His Met Ala Asp Pro Lys Val Pro Lys  
65 70 75 80

Ala Val Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Met Leu Tyr Gln  
85 90 95

Asp Ser Asp Lys Asn Val Ile Leu Arg His Tyr Glu Asp Met Val Val  
15 100 105 110

Asp Glu Cys Gly Cys Gly  
115

## (2) INFORMATION FOR SEQ ID NO:9:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 119 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: GDF-9

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..119

-43-

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

	Ser	Phe	Asn	Leu	Ser	Glu	Tyr	Phe	Lys	Gln	Phe	Leu	Phe	Pro	Gln	Asn
1						5				10					15	
	Glu	Cys	Glu	Leu	His	Asp	Phe	Arg	Leu	Ser	Phe	Ser	Gln	Leu	Lys	Trp
5						20				25				30		
	Asp	Asn	Trp	Ile	Val	Ala	Pro	His	Arg	Tyr	Asn	Pro	Arg	Tyr	Cys	Lys
						35				40				45		
	Gly	Asp	Cys	Pro	Arg	Ala	Val	Arg	His	Arg	Tyr	Gly	Ser	Pro	Val	His
						50				55				60		
10	Thr	Met	Val	Gln	Asn	Ile	Ile	Tyr	Glu	Lys	Leu	Asp	Pro	Ser	Val	Pro
						65			70			75			80	
	Arg	Pro	Ser	Cys	Val	Pro	Gly	Lys	Tyr	Ser	Pro	Leu	Ser	Val	Leu	Thr
						85			90			95				
15	Ile	Glu	Pro	Asp	Gly	Ser	Ile	Ala	Tyr	Lys	Glu	Tyr	Glu	Asp	Met	Ile
						100				105				110		
	Ala	Thr	Arg	Cys	Thr	Cys	Arg									
					115											

## (2) INFORMATION FOR SEQ ID NO:10:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 118 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:

(B) CLONE: BMP-2

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..118

-44-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

	Arg	Glu	Lys	Arg	Gln	Ala	Lys	His	Lys	Gln	Arg	Lys	Arg	Leu	Lys	Ser
1						5					10					15
5	Ser	Cys	Lys	Arg	His	Pro	Leu	Tyr	Val	Asp	Phe	Ser	Asp	Val	Gly	Trp
						20			25						30	
	Asn	Asp	Trp	Ile	Val	Ala	Pro	Pro	Gly	Tyr	His	Ala	Phe	Tyr	Cys	His
						35			40						45	
10	Gly	Glu	Cys	Pro	Phe	Pro	Leu	Ala	Asp	His	Leu	Asn	Ser	Thr	Asn	His
						50			55					60		
	Ala	Ile	Val	Gln	Thr	Leu	Val	Asn	Ser	Val	Asn	Ser	Lys	Ile	Pro	Lys
						65			70				75		80	
	Ala	Cys	Cys	Val	Pro	Thr	Glu	Leu	Ser	Ala	Ile	Ser	Met	Leu	Tyr	Leu
						85			90					95		
15	Asp	Glu	Asn	Glu	Lys	Val	Val	Leu	Lys	Asn	Tyr	Gln	Asp	Met	Val	Val
						100			105					110		
	Glu	Gly	Cys	Gly	Cys	Arg										
						115										

(2) INFORMATION FOR SEQ ID NO:11:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 118 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:

- (B) CLONE: BMP-4

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..118

-45-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Lys Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys  
1 5 10 15

Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp  
5 20 25 30

Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His  
35 40 45

Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His  
50 55 60

10 Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys  
65 70 75 80

Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu  
85 90 95

15 Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val  
100 105 110

Glu Gly Cys Gly Cys Arg  
115

(2) INFORMATION FOR SEQ ID NO:12:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 119 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: Vgr-1

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..119

-46-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser Arg Gly Ser Gly Ser Ser Asp Tyr Asn Gly Ser Glu Leu Lys Thr  
1 5 10 15

Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp  
5 20 25 30

Gln Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp  
35 40 45

Gly Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His  
50 55 60

10 Ala Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro  
65 70 75 80

Lys Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr  
85 90 95

Phe Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val  
15 100 105 110

Val Arg Ala Cys Gly Cys His  
115

(2) INFORMATION FOR SEQ ID NO:13:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 119 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:

(B) CLONE: OP-1

(ix) FEATURE:

(A) NAME/KEY: Protein  
(B) LOCATION: 1..119

-47-

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser Ser Asp Gln Arg Gln  
1 5 10 15

Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp  
5 20 25 30

Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr Tyr Cys Glu  
35 40 45

Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala Thr Asn His  
50 55 60

10 Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Glu Thr Val Pro  
65 70 75 80

Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser Val Leu Tyr  
85 90 95

15 Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val  
100 105 110

Val Arg Ala Cys Gly Cys His  
115

## (2) INFORMATION FOR SEQ ID NO:14:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 119 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: BMP-5

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..119

-48-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Ser Arg Met Ser Ser Val Gly Asp Tyr Asn Thr Ser Glu Gln Lys Gln  
1 5 10 15

Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp  
5 20 25 30

Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp  
35 40 45

Gly Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His  
50 55 60

10 Ala Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro  
65 70 75 80

Lys Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr  
85 90 95

Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val  
15 100 105 110

Val Arg Ser Cys Gly Cys His  
115

(2) INFORMATION FOR SEQ ID NO:15:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 119 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: OP-2

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..119

-49-

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

	Arg	Leu	Pro	Gly	Ile	Phe	Asp	Asp	Val	His	Gly	Ser	His	Gly	Arg	Gln
1						5				10					15	
5	Val	Cys	Arg	Arg	His	Glu	Leu	Tyr	Val	Ser	Phe	Gln	Asp	Leu	Gly	Trp
						20				25					30	
	Leu	Asp	Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	Ala	Tyr	Tyr	Cys	Glu
						35				40					45	
10	Gly	Glu	Cys	Ser	Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	Ala	Thr	Asn	His
						50				55					60	
15	Ala	Ile	Leu	Gln	Ser	Leu	Val	His	Leu	Met	Lys	Pro	Asn	Ala	Val	Pro
						65				70					75	
	Lys	Ala	Cys	Cys	Ala	Pro	Thr	Lys	Leu	Ser	Ala	Thr	Ser	Val	Leu	Tyr
						85				90					95	
20	Tyr	Asp	Ser	Ser	Asn	Asn	Val	Ile	Leu	Arg	Lys	Ala	Arg	Asn	Met	Val
						100				105					110	
	Val	Lys	Ala	Cys	Gly	Cys	His									
						115										

## (2) INFORMATION FOR SEQ ID NO:16:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 120 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:

(B) CLONE: BMP-3

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..120

-50-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Glu Gln Thr Leu Lys Lys Ala Arg Arg Lys Gln Trp Ile Glu Pro Arg  
1 5 10 15

Asn Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp  
5 20 25 30

Ser Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser  
35 40 45

Gly Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His  
50 55 60

10 Ala Thr Ile Gln Ser Ile Val Arg Ala Val Gly Val Val Pro Gly Ile  
65 70 75 80

Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu  
85 90 95

15 Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met  
100 105 110

Thr Val Glu Ser Cys Ala Cys Arg  
115 120

(2) INFORMATION FOR SEQ ID NO:17:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 116 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:

(B) CLONE: MIS

(ix) FEATURE:

(A) NAME/KEY: Protein  
(B) LOCATION: 1..116

-51-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Gly Pro Gly Arg Ala Gln Arg Ser Ala Gly Ala Thr Ala Ala Asp Gly  
1 5 10 15

Pro Cys Ala Leu Arg Glu Leu Ser Val Asp Leu Arg Ala Glu Arg Ser  
5 20 25 30

Val Leu Ile Pro Glu Thr Tyr Gln Ala Asn Asn Cys Gln Gly Val Cys  
35 40 45

Gly Trp Pro Gln Ser Asp Arg Asn Pro Arg Tyr Gly Asn His Val Val  
50 55 60

10 Leu Leu Leu Lys Met Gln Ala Arg Gly Ala Ala Leu Ala Arg Pro Pro  
65 70 75 80

Cys Cys Val Pro Thr Ala Tyr Ala Gly Lys Leu Leu Ile Ser Leu Ser  
85 90 95

15 Glu Glu Arg Ile Ser Ala His His Val Pro Asn Met Val Ala Thr Glu  
100 105 110

Cys Gly Cys Arg  
115

(2) INFORMATION FOR SEQ ID NO:18:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 122 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: Inhibin-alpha

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..122

-52-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ala Leu Arg Leu Leu Gln Arg Pro Pro Glu Glu Pro Ala Ala His Ala  
1 5 10 15

Asn Cys His Arg Val Ala Leu Asn Ile Ser Phe Gln Glu Leu Gly Trp  
5 20 25 30

Glu Arg Trp Ile Val Tyr Pro Pro Ser Phe Ile Phe His Tyr Cys His  
35 40 45

Gly Gly Cys Gly Leu His Ile Pro Pro Asn Leu Ser Leu Pro Val Pro  
50 55 60

10 Gly Ala Pro Pro Thr Pro Ala Gln Pro Tyr Ser Leu Leu Pro Gly Ala  
65 70 75 80

Gln Pro Cys Cys Ala Ala Leu Pro Gly Thr Met Arg Pro Leu His Val  
85 90 95

Arg Thr Thr Ser Asp Gly Gly Tyr Ser Phe Lys Tyr Glu Thr Val Pro  
15 100 105 110

Asn Leu Leu Thr Gln His Cys Ala Cys Ile  
115 120

(2) INFORMATION FOR SEQ ID NO:19:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 121 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: Inhibin-beta-A

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..121

-53-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

	Arg	Arg	Arg	Arg	Arg	Gly	Leu	Glu	Cys	Asp	Gly	Lys	Val	Asn	Ile	Cys
1							5					10				15
5																
	Cys	Lys	Lys	Gln	Phe	Phe	Val	Ser	Phe	Lys	Asp	Ile	Gly	Trp	Asn	Asp
							20					25				30
	Trp	Ile	Ile	Ala	Pro	Ser	Gly	Tyr	His	Ala	Asn	Tyr	Cys	Glu	Gly	Glu
							35				40					45
	Cys	Pro	Ser	His	Ile	Ala	Gly	Thr	Ser	Gly	Ser	Ser	Leu	Ser	Phe	His
					50				55				60			
10																
	Ser	Thr	Val	Ile	Asn	His	Tyr	Arg	Met	Arg	Gly	His	Ser	Pro	Phe	Ala
					65			70				75				80
	Asn	Leu	Lys	Ser	Cys	Cys	Val	Pro	Thr	Lys	Leu	Arg	Pro	Met	Ser	Met
							85			90					95	
15																
	Leu	Tyr	Tyr	Asp	Asp	Gly	Gln	Asn	Ile	Ile	Lys	Lys	Asp	Ile	Gln	Asn
							100			105					110	
	Met	Ile	Val	Glu	Glu	Cys	Gly	Cys	Ser							
				115					120							

(2) INFORMATION FOR SEQ ID NO:20:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 120 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:

- (B) CLONE: Inhibin-beta-B

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..120

-54-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Arg Ile Arg Lys Arg Gly Leu Glu Cys Asp Gly Arg Thr Asn Leu Cys  
1 5 10 15

Cys Arg Gln Gln Phe Phe Ile Asp Phe Arg Leu Ile Gly Trp Asn Asp  
5 20 25 30

Trp Ile Ile Ala Pro Thr Gly Tyr Tyr Gly Asn Tyr Cys Glu Gly Ser  
35 40 45

Cys Pro Ala Tyr Leu Ala Gly Val Pro Gly Ser Ala Ser Ser Phe His  
50 55 60

10 Thr Ala Val Val Asn Gln Tyr Arg Met Arg Gly Leu Asn Pro Gly Thr  
65 70 75 80

Val Asn Ser Cys Cys Ile Pro Thr Lys Leu Ser Thr Met Ser Met Leu  
85 90 95

Tyr Phe Asp Asp Glu Tyr Asn Ile Val Lys Arg Asp Val Pro Asn Met  
15 100 105 110

Ile Val Glu Glu Cys Gly Cys Ala  
115 120

(2) INFORMATION FOR SEQ ID NO:21:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 118 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: Nodal

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..118

-55-

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

	Gly	Trp	Gly	Arg	Arg	Gln	Arg	Arg	His	His	Leu	Pro	Asp	Arg	Ser	Gln	
1						5					10					15	
	Leu	Cys	Arg	Arg	Val	Lys	Phe	Gln	Val	Asp	Phe	Asn	Leu	Ile	Gly	Trp	
5					20				25						30		
	Gly	Ser	Trp	Ile	Ile	Tyr	Pro	Lys	Gln	Tyr	Asn	Ala	Tyr	Arg	Cys	Glu	
						35			40						45		
	Gly	Glu	Cys	Pro	Asn	Pro	Val	Gly	Glu	Glu	Phe	His	Pro	Thr	Asn	His	
					50				55						60		
10	Ala	Tyr	Ile	Ile	Gln	Ser	Leu	Leu	Lys	Arg	Tyr	Gln	Pro	His	Arg	Val	Pro
					65				70			75				80	
	Ser	Thr	Cys	Cys	Ala	Pro	Val	Lys	Thr	Lys	Pro	Leu	Ser	Met	Leu	Tyr	
					85				90						95		
15	Val	Asp	Asn	Gly	Arg	Val	Leu	Leu	Glu	His	His	Lys	Asp	Met	Ile	Val	
						100			105						110		
	Glu	Glu	Cys	Gly	Cys	Leu											
					115												

## (2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 114 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:

(B) CLONE: TGF-beta-1

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..114

-56-

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Arg Arg Ala Leu Asp Thr Asn Tyr Cys Phe Ser Ser Thr Glu Lys Asn  
1 5 10 15

Cys Cys Val Arg Gln Leu Tyr Ile Asp Phe Arg Lys Asp Leu Gly Trp  
5 20 25 30

Lys Trp Ile His Glu Pro Lys Gly Tyr His Ala Asn Phe Cys Leu Gly  
35 40 45

Pro Cys Pro Tyr Ile Trp Ser Leu Asp Thr Gln Tyr Ser Lys Val Leu  
50 55 60

10 Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala Ala Pro Cys Cys  
65 70 75 80

Val Pro Gln Ala Leu Glu Pro Leu Pro Ile Val Tyr Tyr Val Gly Arg  
85 90 95

15 Lys Pro Lys Val Glu Gln Leu Ser Asn Met Ile Val Arg Ser Cys Lys  
100 105 110

Cys Ser

## (2) INFORMATION FOR SEQ ID NO:23:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 114 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:

- (B) CLONE: TGF-beta-2

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..114

-57-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Arg Ala Leu Asp Ala Ala Tyr Cys Phe Arg Asn Val Gln Asp Asn  
1 5 10 15

Cys Cys Leu Arg Pro Leu Tyr Ile Asp Phe Lys Arg Asp Leu Gly Trp  
5 20 25 30

Lys Trp Ile His Glu Pro Lys Gly Tyr Asn Ala Asn Phe Cys Ala Gly  
35 40 45

Ala Cys Pro Tyr Leu Trp Ser Ser Asp Thr Gln His Ser Arg Val Leu  
50 55 60

10 Ser Leu Tyr Asn Thr Ile Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys  
65 70 75 80

Val Ser Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Ile Gly Lys  
85 90 95

15 Thr Pro Lys Ile Glu Gln Leu Ser Asn Met Ile Val Lys Ser Cys Lys  
100 105 110

Cys Ser

(2) INFORMATION FOR SEQ ID NO:24:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 114 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:

- (B) CLONE: TGF-beta-3

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..114

-58-

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Lys Arg Ala Leu Asp Thr Asn Tyr Cys Phe Arg Asn Leu Glu Glu Asn  
1 5 10 15

Cys Cys Val Arg Pro Leu Tyr Ile Asp Phe Arg Gln Asp Leu Gly Trp  
5 20 25 30

Lys Trp Val His Glu Pro Lys Gly Tyr Tyr Ala Asn Phe Cys Ser Gly  
35 40 45

Pro Cys Pro Tyr Leu Arg Ser Ala Asp Thr Thr His Ser Thr Val Leu  
50 55 60

10 Gly Leu Tyr Asn Thr Leu Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys  
65 70 75 80

Val Pro Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Val Gly Arg  
85 90 95

15 Thr Pro Lys Val Glu Gln Leu Ser Asn Met Val Val Lys Ser Cys Lys  
100 105 110

Cys Ser

## (2) INFORMATION FOR SEQ ID NO:25:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 115 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: Human GDF-10

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..115

-59-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Lys Ala Arg Arg Lys Gln Trp Asp Glu Pro Arg Val Cys Ser Arg Arg  
1 5 10 15

Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Asn Glu Trp Ile Ile  
5 20 25 30

Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ala Gly Ala Cys Glu Phe  
35 40 45

Pro Met Pro Lys Ile Val Arg Pro Ser Asn His Ala Thr Ile Gln Ser  
50 55 60

10 Ile Val Arg Ala Val Gly Ile Ile Pro Gly Ile Pro Glu Pro Cys Cys  
65 70 75 80

Val Pro Asp Lys Met Asn Ser Leu Gly Val Leu Phe Leu Asp Glu Asn  
85 90 95

Arg Asn Val Val Leu Lys Val Tyr Pro Asn Met Ser Val Asp Thr Cys  
15 100 105 110

Ala Cys Arg  
115

(2) INFORMATION FOR SEQ ID NO:26:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 115 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: Murine GDF-10

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..115

-60-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Lys Ala Arg Arg Lys Gln Trp Asp Glu Pro Arg Val Cys Ser Arg Arg  
1 5 10 15

5 ~~¶~~ Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Asn Glu Trp Ile Ile  
20 25 30

Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ala Gly Ala Cys Glu Phe  
35 40 45

Pro Met Pro Lys Ile Val Arg Pro Ser Asn His Ala Thr Ile Gln Ser  
50 55 60

10 Ile Val Arg Ala Val Gly Ile Val Pro Gly Ile Pro Glu Pro Cys Cys  
65 70 75 80

Val Pro Asp Lys Met Asn Ser Leu Gly Val Leu Phe Leu Asp Glu Asn  
85 90 95

15 Arg Asn Ala Val Leu Lys Val Tyr Pro Asn Met Ser Val Glu Thr Cys  
100 105 110

Ala Cys Arg  
115

**CLAIMS**

1. Substantially pure growth differentiation factor-10 (GDF-10) and functional fragments thereof.
2. An isolated polynucleotide sequence encoding the GDF-10 polypeptide of claim 1.
3. The polynucleotide sequence of claim 2, wherein the polynucleotide is isolated from a mammalian cell.
4. The polynucleotide of claim 3, wherein the mammalian cell is selected from the group consisting of mouse, rat, and human cell.
5. An expression vector including the polynucleotide of claim 2.
6. The vector of claim 5, wherein the vector is a plasmid.
7. The vector of claim 5, wherein the vector is a virus.
8. A host cell stably transformed with the vector of claim 5.
9. The host cell of claim 8, wherein the cell is prokaryotic.
10. The host cell of claim 8, wherein the cell is eukaryotic.
11. Antibodies reactive with the polypeptide of claim 1 or fragments thereof.

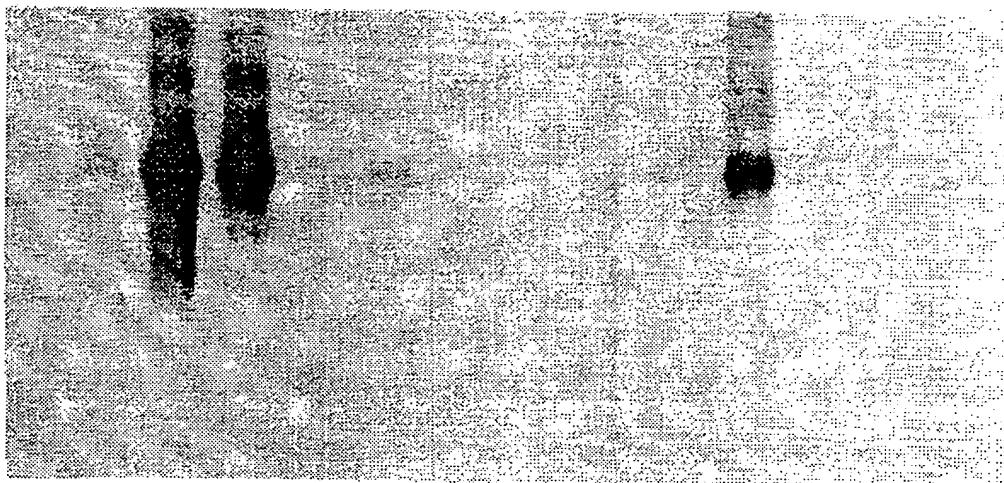
12. The antibodies of claim 11, wherein the antibodies are polyclonal.
13. The antibodies of claim 11, wherein the antibodies are monoclonal.
14. A method of detecting a cell proliferative disorder comprising contacting the antibody of claim 11 with a specimen of a subject suspected of having a GDF-10 associated disorder and detecting binding of the antibody.
15. The method of claim 14, wherein the cell is a uterine cell.
16. The method of claim 14, wherein the cell is a fat cell.
17. The method of claim 14, wherein the detecting is *in vivo*.
18. The method of claim 17, wherein the antibody is detectably labeled.
19. The method of claim 18, wherein the detectable label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, and an enzyme.
20. The method of claim 14, wherein the detection is *in vitro*.
21. The method of claim 20, wherein the antibody is detectably labeled.

22. The method of claim 21, wherein the label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemoluminescent compound and an enzyme.
23. A method of treating a cell proliferative disorder associated with expression of GDF-10, comprising contacting the cells with a reagent which suppresses the GDF-10 activity.
24. The method of claim 23, wherein the reagent is an anti-GDF-10 antibody.
25. The method of claim 23, wherein the reagent is a GDF-10 antisense sequence.
26. The method of claim 23, wherein the cell is a uterine cell.
27. The method of claim 23, wherein the cell is a fat cell.
28. The method of claim 23, wherein the reagent which suppresses GDF-10 activity is introduced to a cell using a vector.
29. The method of claim 28, wherein the vector is a colloidal dispersion system.
30. The method of claim 29, wherein the colloidal dispersion system is a liposome.

31. The method of claim 30, wherein the liposome is essentially target specific.
32. The method of claim 31, wherein the liposome is anatomically targeted.
33. The method of claim 31, wherein the liposome is mechanistically targeted.
34. The method of claim 33, wherein the mechanistic targeting is passive.
35. The method of claim 33, wherein the mechanistic targeting is active.
36. The method of claim 35, wherein the liposome is actively targeted by coupling with a moiety selected from the group consisting of a sugar, a glycolipid, and a protein.
37. The method of claim 36, wherein the protein moiety is an antibody.
38. The method of claim 28, wherein the vector is a virus.
39. The method of claim 38, wherein the virus is an RNA virus.
40. The method of claim 39, wherein the RNA virus is a retrovirus.
41. The method of claim 40, wherein the retrovirus is essentially target specific.

42. The method of claim 41, wherein the moiety for target specificity is encoded by a polynucleotide inserted into the retroviral genome.
43. The method of claim 42, wherein the moiety for target specificity is selected from the group consisting of a sugar, a glycolipid, and a protein.
44. The method of claim 43, wherein the protein is an antibody.

ovary  
liver  
uterus  
fat  
testis  
spleen  
intestine  
pancreas  
seminal vesicle  
kidney  
brain  
thymus  
lung  
heart



**FIG. 1**

1	TGGGGTCATCCGGGCTGTCGGAGTCCCCAGGGACAACTCCAGCCGGGAGCAGGGTGCAC	60
61	AGCCAACTGAGCCCTCCTTGTCTCTGGGCTCAGACCCCTTCACCAACCGTTACT	120
121	CAGCCATGGCTCAGGTCTCTGGATCAAGCTTGGGATCAGCTGGCTCATGGTC	180
181	CGCTGCTCTGGCTGGGGGGGGAGGCTGGGGCACAGGGGGGGCATGGTCCCTCAT	240
241	L L L R G A G C H R G P S W S L	300
241	PGSAAAGLQLQGDDRSQGSPGDA	300
301	CAGCAGGGCGCTCTGGGCCCCAGGACATGGTGCCTATCCACATGCTCAGGCTCT	360
361	A A A L G P G A Q D M V A I H M L R L Y	420
361	ATGAGAACTAACCGAAGGAGGTGCTCCACCCGGAGGGCAACACCGTCCGAAGCTTCC	420
421	B K Y N R R G A P P G G N T V R S F R	480
421	GTGCCCGGGCTGGAAATGATCGACCAAAAGCCCTGTGTATTCTCAACTTGACTTCATGC	480
481	A R L E M I D Q K P V Y F F N L T S M Q	540
481	AAGACTCAGAAATGATCCCTCACAGCCGGCTTCCACTTCTACTCAGAACCTCCACGGTGGC	540
541	D S E M I L T A A P H F Y S E P P R W P	600
541	CCCCGGCTGGTAGGTATCTGCAAGCCCCGAGCTAACAGAACGGCATCCTGGGGCTCTGA	600
601	R A G E V F C K P R A K N A S C R L L T	660
601	CCCCAGGGCTGGCTGGCACCTAATCTTCCGGAGTCTTCCCAGAACACGGCCA	660
661	P G L P A R L H L I F R S L S Q N T A T	720
661	CTCAGGGGCTGGTCCGGGGCCATGGGCTCTGACGGCTCACCACGTCGGCTGTGGCAGG	720
721	Q G L L R G A M A L T P P R G L W Q A	780
721	CCAAGGACATCTCCTCAATCATCAAGGCTGGGGAGATGGAGAGCTGCTCTCTG	780
781	K D I S S I I K A A R R D G E L L S A	840
781	CTCAGCTGGATACTGGGAGAACGGACCCCCGGAGTGCCACGGCCACATGCCCT	840
841	Q L D T G E K D P G V P R P S S H M P Y	900
841	ATATCCTTGTCTAGCCCAATGACCTGGCCATCTCGAACCCAAACAGTGTAGCTGCG	900
901	I L V Y A N D L A I S E P N S V A V S L	960
901	TACAGAGATAACGACCCATTCCAGCTGGAGACTTTGAGCCCTGGAGCCCCAACAGCT	960
961	Q R Y D P F P A G D F E P G A A P N S S	1020
961	CAGCTGATCCCCGGCTGGCAGGGGGCTCAAGGTCTCAAACCCCTGCAAGACAATGAA	1020
961	ADP R V R R A A Q V S K P L Q D N E L	1020

FIG. 2A

1021 TGGCGGGCTGGATGAAAGACCAAGCCAGGGCCTGCATGCCAGAATTCCACAAGCAGC 1080 A  
 P G L D E R P A P A L H A Q N F H K H E  
 1081 AGTTCTGGTCCAGTCCTTCCGGGCACTGAAACCCGCCACGGGGCAAAGAACCCGAAGA 1140  
 F W S S P F R A L K P R T A R K D R K K  
 1141 AGAAGGACCGAACATCACCAGCCCTCTCAAGGTGGACTTGTGAGGAAGA 1200  
 K D Q D T F T A A S S Q V L D F D E K T  
 1201 CGATGAGAAAGCCAGGGGGCACTGGGATGAGCCCCGGTCTGCTCCAGGAGGTACC 1260  
 M Q K A R R R Q W D E P R V C S R R Y L  
 1261 TGAAGCTGGATTGGAGACATGGGATCATCTCTCCAAATCCTTTG 1320  
 K V D F A D I G W N E W I I S P K S F D  
 1321 ACGCCACTACTGTGCTGGGCTGTGGAGTCCCCATGCCAAGATTGTCCGGCCATCCA 1380  
 A Y Y C A G A C E F P M P K I V R P S N  
 1381 ACCATGCCACCATCCAGAGCATGGTCAGGGTGTGGCATTGTCCTGGCATCCAGAGC 1440  
 H A T I Q S I V R A V G I V P G I P E P  
 1441 CATGGCTGTTCCAGACAGATGAACCTCCCTGGAGTCCTCTGGATGAAATCGGA 1500  
 C C V P D K M N S L G V L F L D E N R N  
 1501 ATGGCGTTCTGAAGGGTGTACCCCAATATGTCCTGGTAGAGACCTGTGCCCTGTCCGTAAGATG 1560  
 A V L K V Y P [N M S] V E T C A C R \*  
 1561 GCTTCAGATGAAAGACAGACCTGCTTCATCCCCCTGGCCCTGGAGACTCTGGAGC 1620  
 1621 CAGGGACTTGACTCGGGAGGTTCAAGGTGTTAGACAGAGCTAACGTTACAGGCAAGCCCTGCTGG 1680  
 1681 GACCAAGAAAGATCTGCCACCAATCGCAATTCTTCAGTTCTCCGTGCTGGTAGC 1740  
 1741 TCTGTAAAGACGTGTTGAGTTCTGGAAAGAAATCTGAAATTAACTGTGGCTGCCATTTC 1800  
 1801 CCCATCATCCCTGCCACACTTCAAGGCTCTAGAAATAACGTTGTCCTCAAATGTCAA 1860  
 1861 CTCCAGGCATTGTCCTCTCAAAACCTAGAAAGACTATGCCAAATTCTGGGTACTCCCC 1920  
 1921 CCCCCATGGCAGTTAAATGCTGTTAAACCCCTCAAGGCTGCAATTCTAGAAACGGCC 1980  
 1981 TAACCCATGGCACCGAGTGTAGTATTCTCTAGTTTAATATGTTTCTATTACATG 2040  
 2041 CAGTATGGCACATGTAATCAGGGTTGATTTCCTCTTTAATATGTTTCTATTTC 2100  
 2101 GCAAAACGGAGAGAGTCCATGCCCATCCCCCTGGAGGTTAGGTGTGGG 2160  
 2161 TTGGTCTAAGCATGGTATGGAAATAATACATACAGTAATATGCTTAAACTAAAAGT 2220  
 2221 AACCAAGATTATATTTGTAAATTATACCTTGTATACTGTAGATTGTGAGTGTCTG 2280  
 2281 TGTTTTATGGAAAAGCTAATAATTAAAGGTGGGGAGGTATC 2322

FIG. 2B

GDF-10  
GDF-1  
GDF-3  
GDF-9  
BMP-2  
BMP-4  
Vgr-1  
OP-1  
BMP-5  
OP-2  
BMP-3  
MIS  
Inhibin  
Inhibin  
Inhibin  
Nodal  
TGF- $\beta$ 1  
TGF- $\beta$ 2  
TGF- $\beta$ 3

RPRRDAEPVILGGPGCAQRARRLYVSF-REVGWHRWVIAPRGFLANYCOGCCALPVALSGSGPP  
RKRAAISVPKGFCRNFQHRHQFLINF-QDLGWHKWWVIAPKGFMANYCIGEOPFSMTTYLNS---  
SFNLSEYFKOFLFPQNEOELHDFRLSF-SQLKWDNWIVAPHRYNPRYOKGDOPIRAVRHRYGS---  
REKQAKHKQRKRLKSSOKRHSQRAKRNKNORRHSLYVDF-SDVGWNDDWIVAPPGYHAFYCHGEOPFPLADHLLNS---  
KRSPKHHSQRAKRNKNORRHSLYVDF-SDVGWNDDWIVAPPGYQAFYCHGDOPIFPLADHLLNS---  
SFRGSGSSDYNQGSELTKAQKHELYVSF-QDLGWQDWWVIAPKGYDANYCIGEOPFPLNAHMNA---  
LRLMANVAENSSSDQRAQKHELYVSF-RDLGWQDWWVIAPEGYAYYDGECAFPLNNSYMA---  
SRMSSVGDNTSEQKQAKKHELYVSF-RDLGWQDWWVIAPEGYAAFYDGEOSFPLNAHMNA---  
RLPGIFDDVHGSQHGRQVCRRELYVSF-QDLGWLDWWVIAPOQGYSAYYDGEESFPLDSCMNA---  
EOTLKKARRKQWIEPRNOARRYLKVDFF-ADIGWSEWIISPKSFDAYYDGEAQFPMPKSLKPS---  
GPGRQAQRSAGATAADGPOALRELSVDL---RAERSVLIPETYQANNQGVQOGWPQOSDRNPRY---  
ALRLLQRPPEEPAAHANCHRALNISF-QELGWERWIVYPPSFIFHYQHGGCGLHHIPPNLSLPV---  
RRRRRGLECDGKV---NICKQFFVSF-KDIGNNDWVIAPSGYHANYDGEOPSPHIAGTSGSSL---  
RIRKRGLECDGRT---NLCRQQFFIDF-RLIGWNDWVIAPTGYVGNYDGEOSPAYLAGVFGSAS---  
GWGRQRQRHLPDRSQLRRVKFQVDF-NLIGWGSSWIIYPKOYNAYRQDGEOPNPVGEEFHP---  
RRALDTNYCFSSTE-KNCVRQLYIDFRKDLGWK-WIHEPKGYHANFOLGFPYIWSLD---  
KRALDAAYCFRNQO-DNOCLRPLYIDFCKRDLGWK-WIHEPKGYHANFQAGACPYLWSSD---  
KRALDTNYCFRNLE-ENOCVRPLYIDFRODILGWK-WIHEPKGYYANESEGHCPYLPSAD---

**FIG. 3A**

GDF-10	--NHATIQSIVRA-VGIVPGIPEPCCV--PDKMNSLCVLFL-DENRNAVALKVYPNMSVETCA
GDF-1	ALNHAVLRAALMHA--AAPGAADLPCCV--PARLSPISVLFF-DNSDNVVLROYEDMVVDECCCR
GDF-3	-SNYAFMQLMMH---ADPKVPKAVCCV--PTKLSPISMLYQ-DSDKNVILRHYEDMVVDECCCR
GDF-9	-PVHTMVQNIYE--KLDPSVPRPSCV--PGKYSPLSVLTI-EPDGSIAYKEYEDMIATRCR
BMP-2	-TNHAIIVQTLVNS---VNSKIPKACCV--PTELSAISMLYL-DENEKYVVLKNYQDMVVECCCR
BMP-4	-TNHAIIVQTLVNS---VNSSIPKACCV--PTELSAISMLYL-DEYDKVVLKNYQEMVVECCCR
Vgr-1	-TNHAIIVQTLVHL--MNPEYVVPKPCCA--PTKLNAISVLYF-DDNSNVILKYYRNMTVRA
OP-1	-TNHAIIVQTLVHF--INPETVPKPCCA--PTQLNNAISVLYF-DDSSSNVILKYYRNMTVRA
BMP-5	-TNHAIIVQTLVHL--MFPDHVVPKPCCA--PTKLNAISVLYF-DDSSSNVILKYYRNMTVRA
OP-2	-TNHAILQSLVHL--MKPNAVPKACCA--PTKLSATSVLYY-DSSNNVILRKARNMUVKA
BMP-3	--NHATIQSIVRA-VGIVPGIPEPCCV--PEKMSSLSILFF-DENKNVVLKVYPNMTVATECCCR
MIS	-GNHIVVLLKMQA--RGAALARPFCCV--PTAYAGKLLISLEER--ISAHHVPNMTVATECCCR
Inhibin $\alpha$	-PGAPPTPAQPYSDGGSFKYETVPNLLTQHCA
Inhibin $\beta$ A	-SFHSTVINHYRMRGHSPFANLKSCCV--PTKLRLPMSMLYY-DDGQNIIKKDIQNMIVVECCCR
Inhibin $\beta$ B	-SFHTAVVNQYRMRGGLNPCT-VNSCCI--PTKLSTMMSMLYF-DDEYNIVKRDVPMIVVECCCR
Nodal	-TNHAYIQSSLKR--YQPHRVPSTCA--PVKTKPLSMLYV-DNGRVLLEHHKDMIVVECCCR
TGF- $\beta$ 1	-TOYSKVLALYNO--HNPGASAAPCV--PQALEPLPIVYY-VGRRKPKV-EQLSNMIVRSCKS
TGF- $\beta$ 2	-TQHSRVLSLYNT--INPEASASPCCV--SQDLEPLTILYY-IGKTPKI-EQLSNMIVRSCKS
TGF- $\beta$ 3	-TTHSTVGLYNT--LNPEASASPCCV--PQDLEPLTILYY-VGRTPKV-EQLSNMIVRSCKS

**FIG. 3B**

6 / 7

% amino acid  
identity with  
GDF-10

GDF-1	38%
GDF-3	37%
GDF-9	28%
BMP-2	46%
BMP-4	45%
Vgr-1	43%
OP-1	41%
BMP-5	41%
OP-2	39%
BMP-3	83%
MIS	31%
Inhibin $\alpha$	28%
Inhibin $\beta A$	36%
Inhibin $\beta B$	35%
Nodal	40%
TGF- $\beta 1$	30%
TGF- $\beta 2$	30%
TGF- $\beta 3$	29%

## FIG. 4

KARRKQWDEPRVCSRRYLKVDFA DIGWNEWIISP KSF DAYCAGACEFPM  
 ||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:  
 KARRRQWDEPRVCSRRYLKVDFA DIGWNEWIISP KSF DAYCAGACEFPM  
  
 PKIVRPSNHATIQSIVRAVGII PGIP EPC CVPDKMNSLGVLFLDENRN  
 |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:  
 PKIVRPSNHATIQSIVRAVGIVPGIP EPC CVPDKMNSLGVLFLDENRNA  
  
 LKVYPNMSVDTCACR  
 |||||:|||||:  
 LKVYPNMSVETCACR

## FIG. 5

SUBSTITUTE SHEET (RULE 26)

7/7

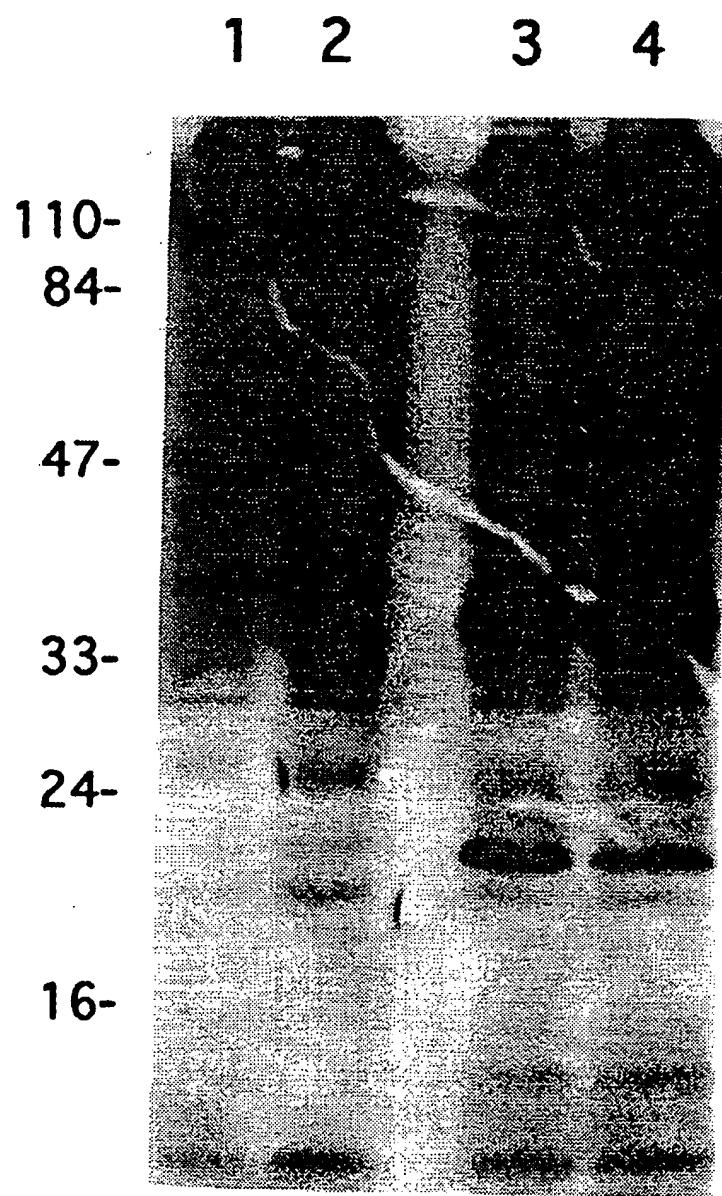


FIG. 6

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/71; C07H 21/00

US CL :530/399; 536/23.5; 435/69.1, 69.4, 320.1, 252.3

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/399; 536/23.5; 435/69.1, 69.4, 320.1, 252.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

GenBank, APS, Dialog

search terms: GDF, endometriosis, uterine, pregnancy, cancer, malignancy

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Proceedings of the National Academy of Sciences USA, Volume 88, issued May 1991, S. Lee, "Expression of growth/differentiation factor 1 in the nervous system: Conservation of a bicistronic structure", pages 4250-4254.	1-10
A	Journal of Biological Chemistry, Volume 268, No. 5, issued 15 February 1993, A.C. McPherron et al., "GDF-3 and GDF-9: Two members of the Transforming Growth Factor- $\beta$ Superfamily Containing a Novel Pattern of Cysteines", pages 3444-3449.	1-10

 Further documents are listed in the continuation of Box C.

See patent family annex.

•	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

Date of mailing of the international search report

02 DECEMBER 1994

JAN 25 1995

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Washington, D.C. 20231

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Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Molecular Endocrinology, Volume 6, No. 11, issued 1992, C.M. Jones et al., "Isolation of <i>Vgr-2</i> , a Novel Member of the Transforming Growth Factor- $\beta$ -Related Gene Family", pages 1961-1968.	1-10
A	Molecular Endocrinology, Volume 4, No. 7, issued 1990, S. Lee, "Identification of a Novel Member (GDF-1) of the Transforming Growth Factor- $\beta$ Superfamily", pages 1034-1039.	1-10

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:  
€
2.  Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-10

**Remark on Protest**  

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-10, drawn to a GDF protein and the DNA encoding the GDF-10 protein.

Group II, claims 11-44, drawn to an antibody and methods of using the antibody.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Groups I and II are drawn to structurally distinct molecules, and although the antibody and GDF-10 protein are related immunochemically, the inventions are considered independent and distinct because they are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

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